WINTER 1993 VOL. 44, No. 4

VIRGINIA JOURNAL OF SCIENCE

OFFICIAL PUBLICATION OF THE VIRGINIA ACADEMY OF SCIENCE

THE VIRGINIA JOURNAL OF SCIENCE

EDITOR:
James H. Martin
Dept. of Biology - PRC
J. Sargeant Reynolds Community College
P.O. Box 85622
Richmond, VA 23285-5622
Phone: (804)371-3064

BUSINESS MANAGER:
William S. Woolcott
Department of Biology
University of Richmond
Richmond, VA 23173
Phone: (804)289-8241

 The Virginia Journal of Science (ISSN:0042-658X) is published four times a year (Spring, Summer, Fall , Winter) by the Virginia Academy of Science, Department of Biology, University of Richmond, Virginia 23173. The pages are electronically mastered in the Parham Road Campus Biology Department of J. Sargeant Reynolds Community College.

Subscription rates for 1993: $27.00 per year, U.S.A.; $35.00 per year, other countries. All foreign remittances must be made in U.S. dollars. Most back issues are available. Prices vary from $5.00 to $25.00 per issue postpaid. Contact the Business Manager for the price of a specific issue.

Changes of address, including both old and new zip codes, should be sent promptly to the following address: Blanton M. Bruner, **Executive Secretary-Treasurer**, Virginia Academy of Science, Department of Biology, University of Richmond, Virginia 23173. All correspondence relating to remittance, subscriptions, missing issues and other business affairs should be addressed to the **Business Manager.**

For instructions to authors, see inside of back cover

VIRGINIA JOURNAL OF SCIENCE
OFFICIAL PUBLICATION OF THE VIRGINIA ACADEMY OF SCIENCE

Vol. 44 No. 4 WINTER, 1993

TABLE OF CONTENTS PAGE

Virginia Journal of Science
Volume 44, Number 4
Winter 1993

Progeny Response And Metabolite Changes In Gestating And Lactating Gilts Fed High Fructose-Corn Syrup[1]

Michael O. Ezekwe[2] and **Ella-Mai V.McBorrough**,
Virginia State University[3], Agricultural Research Station,
Petersburg, VA 23806

ABSTRACT

Pregnant gilts were fed a diet containing 17.9% fructose beginning at d 30 of gestation to study the influence of fructose-corn syrup on serum metabolites, farrowing characteristics and progeny performance. Gilts were bled at 28-d intervals during gestation and on d 14 and 35 of lactation. Diet and stage of gestation affected ($P < 0.01$) serum glucose and fructose levels of the gilts during the treatment period. No significant differences ($P > 0.05$) were observed on serum metabolites and insulin levels during the first 58 days. Serum glucose levels rose significantly ($P < 0.01$) after d 86 of gestation, reaching its peak after 112 d of gestation and remaining high throughout lactation in the fructose-fed group. Dietary fructose did not significantly ($P > 0.05$) influence serum insulin levels. However, the change in insulin levels over the treatment period was significant ($P < 0.05$). Serum triglyceride, free fatty acids and cholesterol were unaffected. Liver weight of progeny at birth and liver glycogen concentrations remained unchanged. Hourly milk yield in gilts, litter size and weight and progeny survival at birth and at d 35 were not affected ($P > 0.05$) by maternal diet. The results indicated that fructose diet increased serum fructose and glucose concentrations only during late gestation and lactation but did not influence the progeny energy reserves or neonatal pig performance.

Key Words: Gilt, gestation, metabolites, progeny, lactation, fructose, diet.

INTRODUCTION

Elevating blood glucose concentrations or sparing glucose in gestating sows can be beneficial to fetal pigs by increasing nutrient supply to the developing pigs for growth, liver glycogen deposition, and/or lipid synthesis. Fetuses and piglets from gilts made diabetic via alloxan or streptozotocin injection showed significant elevation of liver weight, liver glycogen content and body lipid (Ezekwe, et al, 1984; Ezekwe, 1986) as well as demonstrated an improvement in postnatal survival (Kasser, et al., 1982).

---

1 Contibution of Virginia State University. Journal Article Series No. 178.

2 Address all correspondence to: Dr. Michael O. Ezekwe, Virginia State Univerity, Agricultural Research Station, P. O. Box 9122, Petersburg, VA 23806, Telephone: (804) 524-5160, Fax: (804) 524-5186

3 The use of any trade names of vensdrs does not imply approval to the exclusion of other products or vendors that may also be suitable.

Since crystalline fructose and fructose-corn syrup have become available, a number of studies have shown that sows fed fructose showed significant elevation of blood glucose and fructose concentrations, while maintaining lower insulin concentrations (White et al., 1987). Milk yield from these fructose-fed sows increased, and body weight of pigs at weaning were heavier than controls. The authors suggested that the dietary fructose may have served as a precursor for milk constituents and thus spared glucose. However, other reports showed that a fructose diet fed during late gestation and lactation did not influence sow metabolite profile, insulin levels, or milk yield (Coffey et al., 1987; Kveragas, et al., 1988). These studies were conducted during the third trimester of gestation or lactation when the sows' metabolite profile was greatly influenced by gestational or lactational stress. No information is available on the effects of fructose diet on maternal serum metabolites during the first and second trimester of gestation. The objective of this study was to determine the influence of fructose-corn syrup, beginning at d 30 of gestation and throughout lactation, on the gilt blood metabolite and insulin concentrations, milk yield, farrowing performance characteristics, piglet energy reserves, and survival.

MATERIALS AND METHODS

Twelve pregnant crossbred gilts (Duroc x Yorkshire x Landrace) with known breeding dates were assigned to two treatment groups. Gilts were weighed and randomly allotted to control and fructose fed groups, respectively. Animals were fed 1.82 kg/d of corn-soybean meal diet containing 14% protein and adequately fortified with vitamins and minerals to meet the NRC (1979) nutrient requirements for gestating swine (Table 1). The fructose-fed group received the same diet but with 32.5% of the carbohydrate portion replaced with fructose-corn syrup (American Sweeteners Inc., Frazer, PA). The experimental diet was essentially isocaloric and iso-nitrogenous and the fructose diet was formulated to contain 17.9% fructose similar to levels used by White et al; (1984, 1987).

Blood was taken by venapuncture from the anterior vena cava of all the animals before the initiation of treatments and at 28-d intervals until parturition. During lactation, blood was collected from the gilts at d 14 and 35 of lactation. All gilts were meal-fed and blood was collected about 2 h after each feeding. Serum was collected after centrifugation and frozen at -20C until analyzed for glucose, cholesterol and triglycerides (Sigma Tech. Bul. #510, 352 and 405, respectively. Sigma Chemical Co.; St. Louis, MO.). Serum free fatty acids (FFA) were assayed according to methods of Dunocombe (1963, 1964). The lower range of sensitivity for the assay was 10 μEq/L of serum. Serum insulin was determined by double antibody radioimmunoassay techniques using an assay kit supplied by Cambridge Medical Diagnostic (Cambridge Medical Diagnostics, Billerica, MASS.) with a lower range of sensitivity of 5 μU/mL of serum. Intra-assay coefficient of variation was 11%. Serum fructose was assayed according to the resorcinol method of Roe (1954) as modified by Arsenault and Yaphe (1965).

All farrowings were attended. At parturition, pigs were cleaned and weighed and 1 or 2 pigs/litter with or near the average body weight of the litter were mechanically stunned and killed by exsanguination. Blood was collected, serum harvested and frozen for later biochemical determinations. The liver was removed,

TABLE 1. Composition of control and fructose diet[a]

Item	Control Diet g/100g	Fructose Diet g/100g
Corn (IFN 4-02-935)	79.16	48.48
Soybean Meal, 48% CP (IFN 5-04-612)	12.69	18.37
Fructose Corn Syrup[b]	--	32.5
Tallow	1.5	--
Alfafa (IFN 1-00-023)	5.00	5.00
Dicalcium Phosphate (IFN 6-01-080)	1.00	1.00
Limestone (IFN 6-01-069)	0.90	0.90
Trace Mineral Salt[c]	0.50	0.50
Salt (IFN 6-14-013)	0.50	0.50
Vitamin Premix[d]	0.25	0.25
ME, Kcal/kg	3365	3373

[a]Calculated to contain 14% crude protein
[b]American Sweeteners Inc., Frazer, Pa. High fructose corn syrup contains 55% fructose on dry matter basis added to diet to supply 17.9% fructose
[c]Contained (%) 17.5 Zn; 14 Mn; 8.8 Fe; 1.7 Cu; 0.35 I and 0.35 Co
[d]Supplied (per kg of premix) 1.76 g riboflavin; 8.8 g panthothenic acid; 8.8 g niacin; 8.8 mg vitamin A; 176,000 I.U. vitamin D; 4400 I.U. vitamin E; 440 mg menadione dimethylprimidinol bisulfite; 88.2 mg biotin and 40 mg Se

bloted dry and weighed. A piece was cut out in triplicate immediately digested in 30% KOH saturated with Na_2SO_4 for glycogen determination according to methods of Lo et al. The remaining litters were equalized to 7 or 8 pigs and allowed to suckle the dams. Gilts were fed about 5.4 kg/day during lactation and the amount fed was scaled to the number of pigs nursed. Creep feed was not provided to the pigs; however, pigs had access to the sows' diet before weaning. Survival of pigs and body weight at birth and at weaning were recorded. Milk yield was estimated at d 14 of lactation by the weigh-suckle-weigh method (Speer and Cos, 1984).

Statistical evaluation was done by one-way analysis of variance (Steel and Torrie, 1960). A 2 x 6 factorial analysis of variance was used to determine the effect of diet and gestational/lactational stage (time) on sow blood metabolites and insulin. Treatment means were separated by the least significant difference (LSD) technique.

TABLE 2. Reproductive performance and milk yield in fructose-fed and control gilts[a]

CHARACTERISTIC[b]	TREATMENT	
	CONTROL	FRUCTOSE
Gestation Length, d	114.6 ± 0.3	114.4 ± 0.2
Wt. at d 30, kg	138.8 ± 10.8	142.8 ± 8.2
Wt. at d 112, kg	166.8 ± 11.8	175.2 ± 8.1
Wt at d 35 of lactation, kg	155.7 ± 2.1	163.5 ± 9.2
Litter size	10.5 ± 1.2	8.5 ± 0.8
Litter wt at birth, kg	11.2 ± 0.9	10.4 ± 0.53
Litter wt at d 35 of lactation, kg	032.7 ± 5.6	37.2 ± 1.3
Survival at birth, %	90.1 ± 7.5	95.6 ± 2.4
Survival at d 35 of lactation	69.5 ± 9.4	84.1 ± 6.5
Hourly milk yield, g	249.3 ± 60.4	267.6 ± 71.0

[a]Mean ± SEM for 6 animals
[b]Control and fructose groups did not differ ($P > 0.05$)

RESULTS

Table 2 represents the reproductive performance characteristics and hourly milk yield of the fructose-fed and control gilts. There were no differences ($P > 0.05$) in gestation length, gilt weight gain during gestation, or weight at weaning. Litter size and weight at birth were not significantly affected by treatment. Although litter weight at weaning, litter survival (birth and weaning) and milk yield were slightly higher in fructose fed gilts, these trends were not statistically significant ($P > 0.05$).

The effect of dietary fructose during gestation and lactation on blood glucose and fructose concentrations is shown in Figure 1 and 2. Diet and stage of gestation affected ($P < 0.01$) serum glucose and fructose levels during the treatment period. Serum glucose levels rose significantly ($P < 0.01$) after d 86 of gestation, reaching a peak at d 112 of gestation, and remaining high throughout lactation in fructose-fed dams. Fructose levels exhibited elevated concentrations ($P < 0.01$) at d 112 of gestation and throughout lactation in the fructose fed group. Serum glucose and fructose levels in the fructose fed dams were affected ($P < 0.05$) by the stage of gestation (time). There was a diet by stage-of- gestation/lactation interaction ($P < 0.05$) for serum glucose and fructose.

Dietary fructose did not affect ($P > 0.05$) serum insulin levels (Figure 3). However, the change in insulin levels over the treatment period was significant ($P < 0.05$). Cholesterol, triglycerides and free fatty acids were not significantly ($P > 0.05$) influenced by diet (Figures 4, 5 & 6).

Stage of gestation/lactation did not influence ($P > 0.05$) serum triglyceride, cholesterol or free fatty acids levels. Peak levels of triglycerides were reached at d 112 of gestation and decreased by d 14 of lactation (Figure 5). The progeny performance characteristics did not show significant differences between fructose-

TABLE 3. Serum and liver metabolite levels in progeny of fructose-fed and control gilts at birth[a]

ITEM[b]	TREATMENT	
	CONTROL	FRUCTOSE
Glucose, mg/dL	88.3 ± 18.0	106.3 ± 18. 0
Fructose, mg/dL	25.2 ± 9.0	36.0 ± 10.2
Triglycerides, mg/dL	53.1 ± 17.7	79.7 ± 26.6
Free fatty acid, %Eq/L	462.1 ±100.7	333.1 ±100.7
Cholesterol, mg/dL	40.2 ± 3.6	32.5 ± 3.6
Glycogen, mg/g	88.4 ± 12.0	99.8 ± 11.4
Liver wt, g	32.7 ± 3.8	30.9 ± 1.8

[a]Mean ± SEM for 7 piglets
[b]Did not differ ($P > 0.05$)

fed and control pigs (Table 3). Progeny serum metabolites as well as liver weight and liver glycogen concentrations were not influenced significantly ($P > 0.05$) by maternal prepartum diet. There was a trend ($P < 0.06$) toward elevated serum glucose levels in progeny of fructose-fed dams at birth.

DISCUSSION

Results demonstrated that the ability of a fructose diet to alter maternal serum glucose in gestating gilts depended on the stage of gestation. A number of studies involving fructose feeding to gestating and/or lactating sows have shown that a fructose diet increased (White et al., 1984; 1987) or did not affect serum glucose (Coffey et al., 1987; Kveragas et al., 1988). The present study showed that a fructose diet failed to increase maternal serum glucose in early and mid-gestation in gilts, while a significant increase was observed during late gestation and lactation. It is not clear whether the observed response was due primarily to the physiological state of the animal or to the fructose diet *per se*. The high concentration of serum fructose in treated animals during late gestation indicated that carboydrate metabolism was being influence by late gestational physiology. Kveragas et al; (1988) showed no change in mean concentrations of glucose overtime in sow plasma as a result of dietary fructose fed during gestation. In the present study, while fructose diet failed to increase serum glucose during the early and mid gestation, serum glucose was markedly elevated during late gestation in respone to fructose feeding. In both Kveragas et al., (1988) and the present study, blood fructose concentrations were significantly increased. White et al, (1984) showed that maximum glucose stimulation was reached two hours after feeding, suggesting that the time between feeding and blood sampling might affect the concentration of metabolites. The low serum fructose concentrations in control gilts were in agreement with those reported for sows (Randal L'Ecuyer, 1976).

TABLE 3. Serum and liver metabolite levels in progeny of fructose-fed and control gilts at birth[a]

ITEM[b]	TREATMENT	
	CONTROL	FRUCTOSE
Glucose, mg/dL	88.3 ± 18.0	106.3 ± 18.0
Fructose, mg/dL	25.2 ± 9.0	36.0 ± 10.2
Triglycerides, mg/dL	53.1 ± 17.7	79.7 ± 26.6
Free fatty acid, %Eq/L	462.1 ±100.7	333.1 ±100.7
Cholesterol, mg/dL	40.2 ± 3.6	32.5 ± 3.6
Glycogen, mg/g	88.4 ± 12.0	99.8 ± 11.4
Liver wt, g	32.7 ± 3.8	30.9 ± 1.8

[a]Mean ± SEM for 7 piglets
[b]Did not differ ($P > 0.05$)

Significant correlation between glucose and fructose reported by White et al; (1987) suggests that either fructose is converted to glucose in vivo or fructose has a glucose-sparing effect. Numerous studies in humans, calves and pigs showed that only very small amounts of fructose to glucose conversion occurred (Edwards and Powers, 1967; Dunningam and Ford, 1975; Aitken et al., 1972). It is likely that hormones mediate the dietary fructose alteration of carbohydrate metabolism by increasing glucose production and decreasing its clearance from circulation. Burt and Pulliam, (1960) suggested that increased glucocorticoids in late pregnancy might be involved. In humans, a proteolytic enzyme capable of inactivating insulin has been identified in placental membrances (Frienkel and Goodner, 1960). These factors may contribute to insulin resistance and the tendency for insulin to increase (Figure 2) with the increase in glucose concentration often referred to as gestational diabetes. Though diet did not influence serum insulin concentration in the present studies, serum insulin increased ($P < 0.05$) with time during the experiment with increasing glucose concentration. Serum insulin was decreased (White et al, 1984; 1987) or unaffected (Coffey et al., 1987; Kveragas et al, 1988) by fructose diet fed to gestating and/or lactating sows.

The lack of significant differences in reproductive performance characteristics and progeny performance (Table 3) indicates that the observed maternal nutrient alterations may not have reached the critical level needed to alter fetal metabolism. Glucose crosses the placenta by facilitated diffusion against a concentration gradient (Widdas, 1961). When the maternal level of plasma glucose is normal, the "fetal diet" contains a relatively large amount of glucose capable of sustaining 50-70% of fetal oxidative requirements (Battaglia and Meschia, 1978). Milk yields did not differ significantly ($P > 0.05$). Higher milk yield from sows fed fructose during lactation was reported in previous studies by White et al, (1987). On the other hand, Kveragas et al (1988); and Coffey et al., (1987) reported no increases in milk yield when sows were fed fructose during late gestation and lactation.

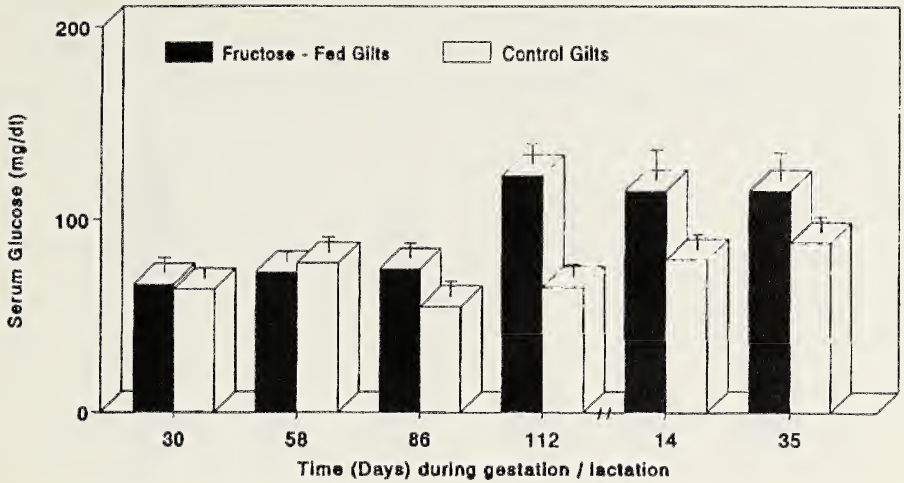

FIGURE 1. Effect of high fructose diet during gestation and lactation on serum glucose concentrations in gilts. Pregnant gilts were fed either control or high fructose (17.9% of diet composition) diet during gestation and lactation. Blood was sampled from the animals prior to initiation of experimental treatments (d 30 of gestation) and subsequently at 28-d intervals and on d 14, and 35 of lactation. ANOVA indicated a significant ($P < 0.01$) effect of diet and stage of gestation/lactation on glucose concentrations during the study. There was a diet by stage of gestation lactation interaction ($P < 0.05$) for serum glucose and fructose of the gilts.

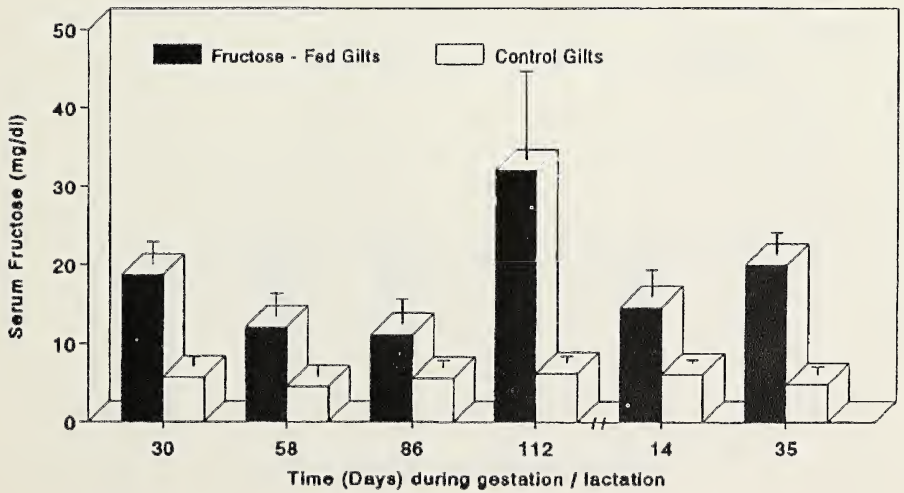

FIGURE 2. Effect of high fructose diet during gestation and lactation on serum fructose concentrations in gilts. ANOVA indicated a significant ($P < 0.01$) effect of diet and stage of gestation/lactation on fructose concentrations during the study. Diet by stage of gestation/lcatation interaction ($P < 0.05$) was observed in the animals.

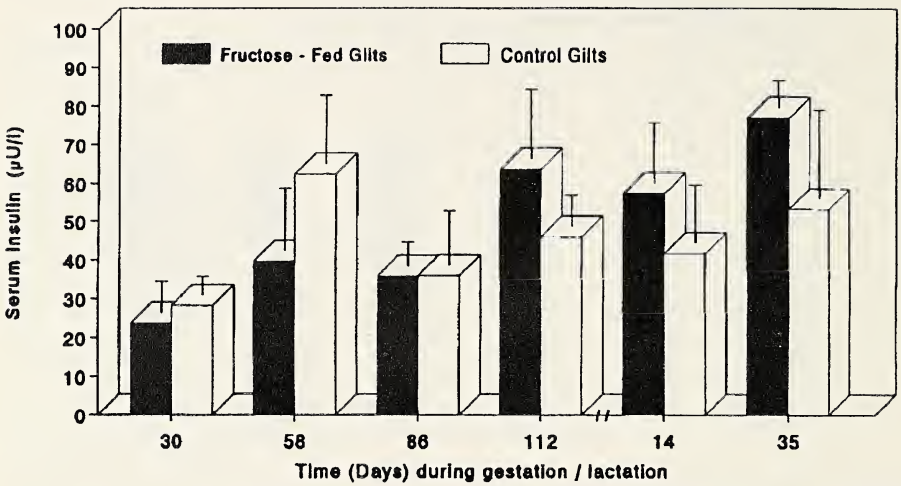

FIGURE 3. Effect of high fructose diet during gestation and lactation on serum insulin concentrations in gilts. ANOVA revealed no significant ($P > 0.05$) effect of diet, however stage of gestation/lactation (time) influenced ($P < 0.05$) serum insulin concentrations.

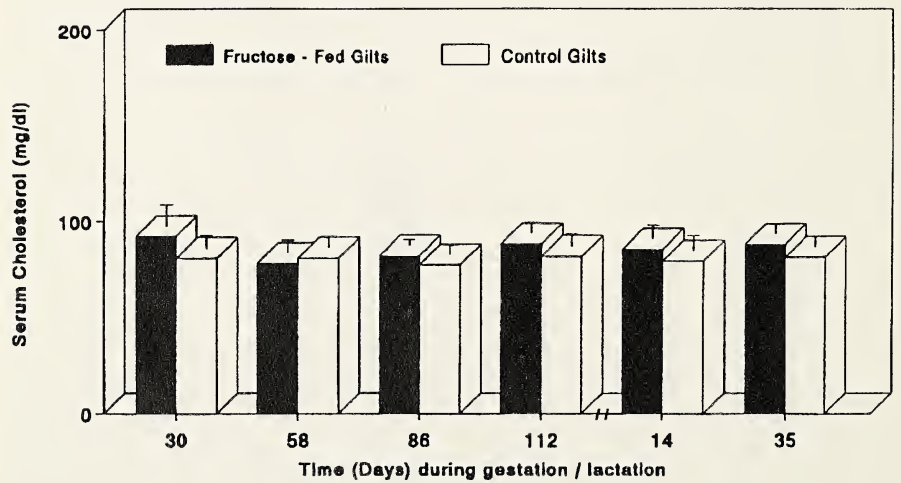

FIGURE 4. Mean concentration of serum cholesterol in gilts fed high fructose and control diet during gestation and lactation. ANOVA indicated no significant effect of diet and stage of gestation/lactation on serum cholesterol levels.

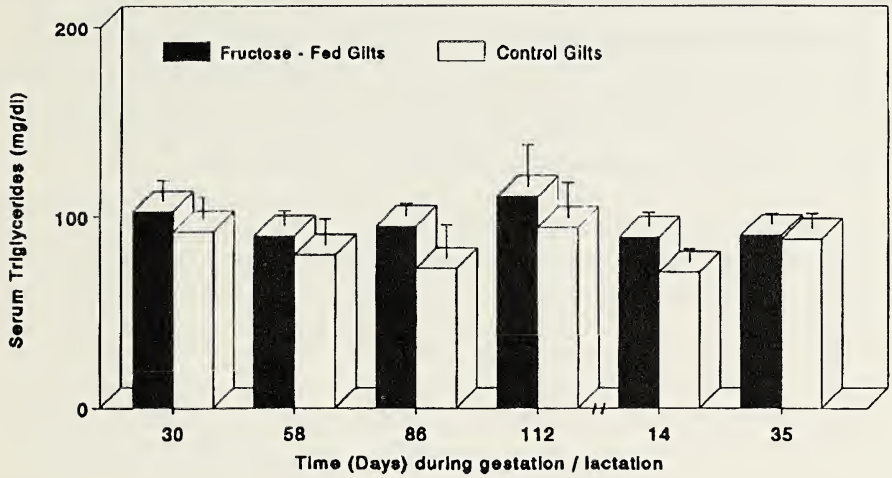

FIGURE 5. Mean serum triglyceride concentrations in gilts fed high fructose and control diet during gestation and lactation. ANOVA showed no significant diet and stage of gestation/lactation effect on serum triglyceride concentrations of gilts.

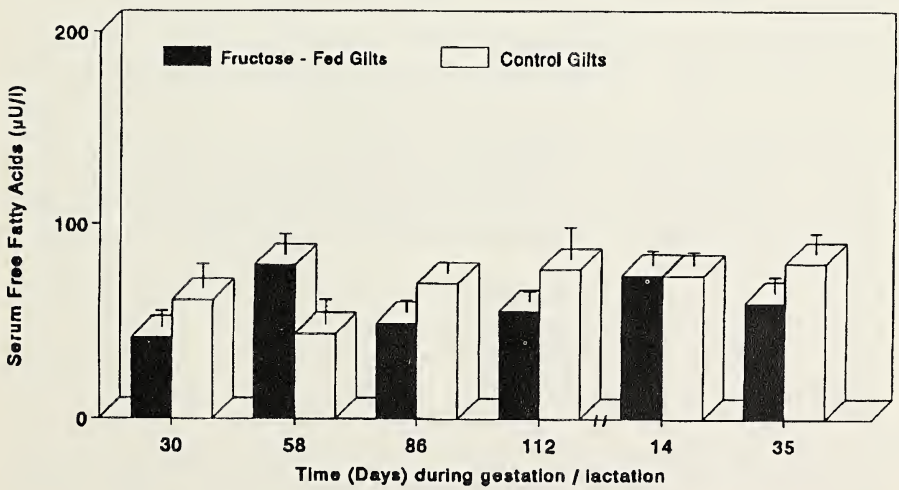

FIGURE 6. Effect of dietary fructose on serum free fatty acids concentrations in gilts during gestation and lactation. ANOVA indicated no significant ($P > 0.05$) effect of diet and stage of gestation/lactation on serum free fatty acids concentrations of the gilts.

Positive correlations between pig weaning weights and sow milk yield have been reported (Boyd et al., 1978).

Although glucose concentrations were high in fructose-fed gilts, we do not know if the level was sustained long enough to affect fetal liver glycogen synthesis. Glycogen storage depends on the availability of sufficient glucose substrate. The fluctuations in cholesterol, FFA and triglycerides may be indicative of normal variations in circulating metabolites rather than treatment effects.

This study showed that extended feeding of fructose diet does not influence the metabolite status of pregnant gilts during early or mid-gestation. While, serum glucose and fructose levels were significantly higher in fructose-fed gilts, no factors related to piglet survival were influenced by maternal diet. The fact that gilts were unresponsive to fructose diet during early and mid-gestation, suggests that factors other than fructose may be responsible for the stimulation of serum glucose and fructose concentrations during late gestation. It would appear that the real predisposing factor was the diabetic-like state which was evident during late gestation and lactation. This study indicated that late gestation was an ideal time to manipulate the sow's metabolism in favor of fetal development.

ACKNOWLEDGMENT

This study was supported by the USDA-CSRS. I greatly appreciate the contributions of Ella-Mai McBorrough in blood collection and analysis, as well as in the initial summary of the data.

LITERATURE CITED

Ezekwe, M. O., Ezekwe, E. I., Sen, D. K. and Ogolla, F. 1984. Effect of maternal streptozotocin-diabetes on fetal growth, energy reserves and body composition of newborn pigs. J. Anim. Sci. 59:974-980.

Ezekwe, M. O. 1986. Effect of streptozotocin induced diabetes in primiparous sows on subsequent reproductive performance. J. Anim. Sci. 62:1052-1011.

Kasser, T. R., Gahagan, J. H. and Martin, R. J. 1982. Fetal hormones and neonatal survival in response to altered maternal serum glucose and free fatty acids concentration in pigs. J. Amin Sci. 55:1351-1359.

Boyd, R. D., Moser, D. B., Peo, Jr., E. R., and Cunningham, P. J. 1978. Effect of energy source prior to parturition and during lactation on tissue lipid, liver glycogen and plasma levels of some metabolites in the newborn pig. J. Anim. Sci. 47:874-882.

Coffey, M. T., Yates, J. A. and Combs, G. E. 1987. Effect of feeding sows fat or fructose during late gestation and lactation. J. Anim. Sci. 65:1249-1256.

White, C. E., Head, H. H., Buchman, K. C. and Bazer, F. W. 1987. Yield and composition of milk and weight gain of nursing pigs from sows fed diets containing fructose or dextrose. J. Anim. Sci. 59:141-150.

White, C. E., Head, H. H., Bazer, F. W. 1984. Response of plasma glucose, fructose and insulin to dietary glucose and fructose in lactating sow. J. Nutr. 114:361-368.

Kveragas, C. L., Seerley, R. W., Martin, R. J. and Vandergrift, W. L. 1988. Maternal feeding of glucose, fructose or fat and its effect on sows and pigs. Nutr. Rep. Int. 37:665-674.

Nutrient Requirements of Swine. 1979. National Research Council, National Academy of Sciences, Washington, D.C. pp. 1-52.

Dunocumbe, W. G. 1963. The colorimetric microdetermination of long chain fatty acids. Biochem. J. 88:7.

Dunocombe, W. B. 1964. The colorimetric microdetermination of nonesterified fatty acids in plasma. Clin. Chim. Acta 9:122-125.

Roe, J. H. 1934. A colorimetric method for the determination of fructose in blood and urine. J. Biol. Chem. 107:15-22.

Arsenault, G. P., and Yaphe, W. 1965. Effect of acetaldehyde, acetic acid and ethonal on the resorcinol test for fructose. Anal. Biochem. 13:133-142.

Lo, S. J., Russel, C. and Taylor, A. W. 1970. Determination of glycogen in small tissue samples. J. Appl. Physiol. 28:234-236.

Speer, V. C. and Cos, D. F. 1984. Estimating milk yield of sows. J. Anim. Sci. 59:1281-1285.

Steel, N. C., Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw Hill, New York, pp. 1-481.

Randal, G. C. B. and L'Ecuyer, C. 1976. Tissue glycogen and blood glucose and fructose levels in pig fetuses during the second half of gestation. Biol. Neonate. 28:74-82.

Edwards, A. V. and Powers, N. 1967. Effect of intravenous infusion of fructose in newborn calfs. Nature (Land). 214:728-729.

Aitken, J. M., Newton, D. A. G., Hall, P. E. and Dinwoodle, A. J. 1972. The corticoid, insulin and growth hormone responses to intravenous fructose in men and women. Acta Med. Scand. Suppl. 524:165-172.

Dunningam, M. G. and Ford, J. A. 1975. The insulin response to intravenous fructose in relations to glucose levels. J. Clin. Endocrinol. Metab. 40:629-636.

Burt, R. L. and L. P. Pulliam 1960. Carbohydrate metabolism in pregnancy. Lactic acid production following administration of insulin. Obstet. Gynecol. 14:518-522.

Frienkel, N. and Goodner, C. J. 1960. Carbohydrate metabolism in pregnancy 1. Metabolism of insulin by human placental tissue. J. Clin. Invest. 39:116-131.

Widdas, W. F. Transport mechanism in foetus. (1961). Brit Med. Bull. 17:107-111.

Battaglia, F. D. and Meschia, G. (1978). Principal substrates of fetal metabolism. Physiol. Rev. 58:499-527.

Virginia Journal of Science
Volume 44, Number 4
Winter 1993

Microzooplankton in the Lower Chesapeake Bay, and the Tidal Elizabeth, James, and York Rivers

Gyung-Soo Park and **Harold G. Marshall**,
Department of Biological Sciences, Old Dominion University,
Norfolk, Virginia 23529-0266

ABSTRACT

Results of a one year study in the lower Chesapeake Bay and three tidal rivers indicate an abundant microzooplankton population, with a mean concentration of 4,231.1/Liter. The most abundant components are the non-loricate ciliates (2,518.2/L, 59.5% of the annual total) and tintinnids (1,400.1/L, 33.1%). In lesser abundance were the rotifers (191.4/L) and nauplii larvae (121.7/L). Seasonal abundance maxima were highest in summer, followed by fall, spring and winter.

INTRODUCTION

Microzooplankton includes those planktonic animals from 20 to 200 μm in size (Sieburth et al., 1978). The microzooplankton represent an essential link in specific food webs and energy transfer steps between the basic trophic levels within estuarine and other aquatic ecosystems (Laval-Peuto et al., 1986). They are considered consumers of picoplankton and nanoplankton in various aquatic habitats and are themselves a common food source for larval fish and other zooplankters (Rassoulzadegan and Sheldon, 1986; Dolan, 1991). Because the microzooplankton possess this strategic position within the trophic structure of estuaries, it is important to know more about this community and specifically its seasonal patterns of abundance in the lower Chesapeake Bay.

The first microzooplankton observations in the Chesapeake Bay were from whole water samples studied by Wolfe et al. (1926), with this same material discussed further by Cowles (1930). Their results included the listing of several protozoa in a mixed category that also contained dinoflagellates. Other early plankton studies in the Bay region by Morse (1947) and Whaley and Taylor (1968), used net collections that contained several groups of microzooplankton (e.g. tintinnids, rotifers, copepod larvae). More recently, Brownlee and Jacobs (1987) discussed both mesozooplankton (>200 μm) and microzooplankton (<200 μm) composition, abundance and biomass in the upper Chesapeake Bay.

Dolan and Coats (1991) studied the vertical distribution of microzooplankton in the upper Chesapeake Bay and noted the ciliate component was dominated by oligotrichs and tintinnids. Dolan (1991), using whole water samples, discussed the ciliate populations in the Chesapeake Bay in relation to their role as consumers. In Back Bay, Virginia, Marshall et al. (1988) conducted a year study of macrozooplankton and microzooplankton (<150 μm). Using whole water samples, they found peak abundance occurred in late spring, with the samples dominated by tintinnids and non-loricate ciliates.

To obtain a more accurate estimate of the composition and abundance of the microzooplankton, several investigators have emphasized the importance of using whole water samples for analysis over net tow samples. For instance, Beers and Stewart (1964) noted a loss of 88% of the total microzooplankton when using a 35 μm mesh net. Those forms likely to be lost are the ciliated protozoans, which have been reported as accounting for >95% of the microzooplankton assemblages (Chang, 1990). In a comparison study on methodology, Brownlee and Jacobs (1987) used a combination of net sizes (44 μm & 20 μm) and whole water samples. Based on the numbers of organisms present in the samples, the two net samples greatly underestimated the total microzooplankton. The percentage retained of the total microzooplankton concentrations in the 44 μm and 20 μm nets were 5% and 26% respectively for a Choptank River sample and only 2% and 4% respectively for a Chester River sample. In regard to biomass, the percent loss was 49% and 44% retained by the 44 μm mesh net for the Choptank and Chester Rivers respectively. In their monthly sampling, they based their analysis on collections from a 44 μm mesh net, resulting in counts of "only the larger microzooplankton", with a significant amount of microzooplankton lost through their nets.

The results from earlier cruises that sampled microzooplankton composition in the lower Bay (Wolfe, et al., 1926, Whaley & Taylor, 1968), were incomplete in their coverage of the non-loricate ciliates. This is in contrast to the upper Bay, where these ciliates have been investigated by Brownlee and Jacobs (1987), Dolan and Coats (1991), and Dolan (1991). The present study was undertaken to provide general information on the abundance of microzooplankton in the lower Chesapeake Bay (Marshall, 1993). The specific objective of this report is to present the seasonal abundance patterns of the four major microzooplankton categories at stations in the lower Chesapeake Bay and the tidal James, York and Elizabeth Rivers. The four microzooplankton groups are: tintinnids, non-loricate ciliates, rotifers, and nauplii larvae.

METHODS

Monthly collections were made at 10 stations in the lower Chesapeake Bay and the James, York and Elizabeth Rivers from July 1992 through June 1993 (Figure 1). Whole water samples were taken in order to collect a more accurate representation of the microzooplankton (Brownlee and Jacobs, 1987; Beers and Stewart, 1964; and Chang, 1990). Two 15 liter carboys were filled on station, with composite water samples, taken from a vertical series of 5 depths above the pycnocline at 4 stations in the Chesapeake Bay (WE4.2, LE5.5, CB6.1, CB7.4), and two stations from the James River (TF5.5, RET5.2), the York River (TF4.2, RET4.3) and the Elizabeth River (ER2, ER5). The carboys were thoroughly mixed when filled, and a 1 liter sub-sample was taken from each and preserved with Lugol's solution (10 ml). Each sample was settled for 72 hours in the laboratory before a series of two siphoning and settling steps were taken to obtain a 100 ml concentrate. The analysis process consisted of analyzing three sub-sets from the 100 ml concentrate from each replicate sample. The analysis of three sub-groups is necessary to reduce the problem of silt covering specimens in the samples and to be able to separate the microzooplankters that vary greatly in size and weight. The first step involves removing detritus and larger zooplankton from the sample. This is accomplished

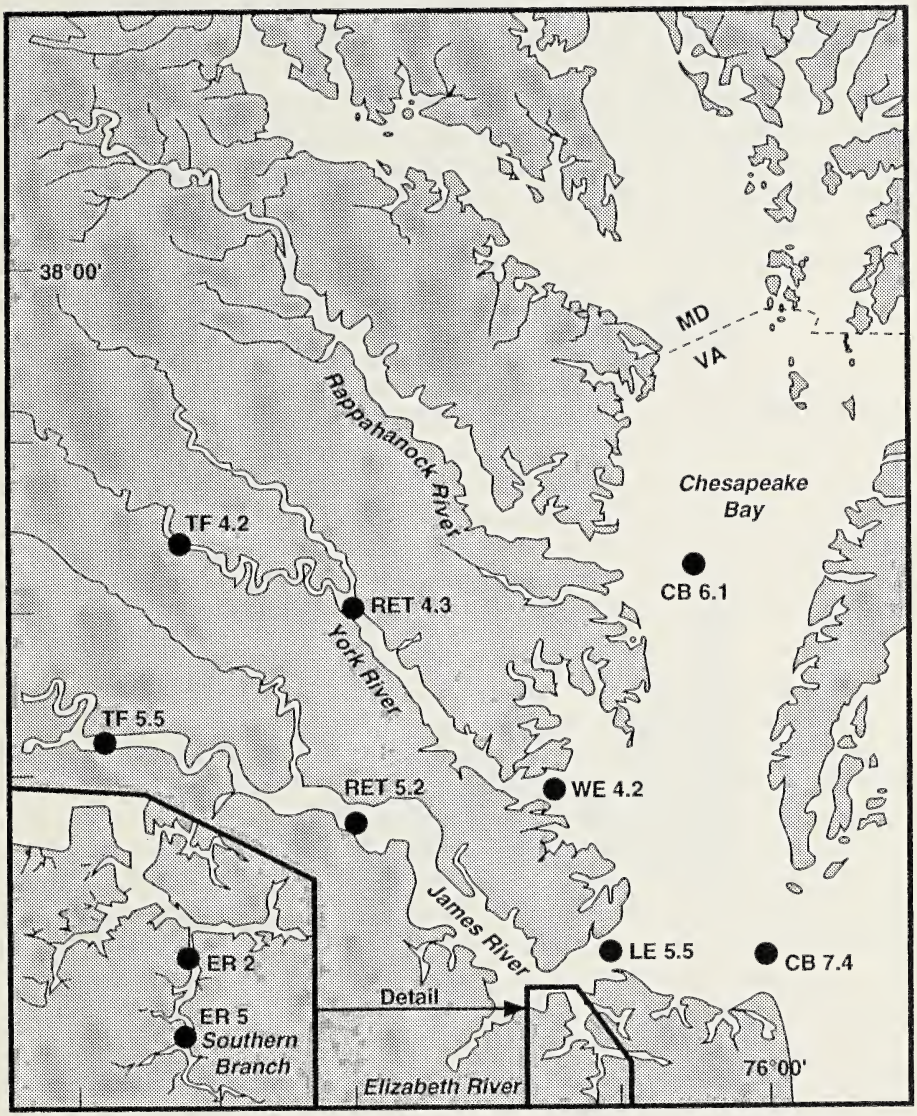

FIGURE 1. Station locations in the Chesapeake Bay, and the Elizabeth, James and York Rivers.

by passing the 100 ml concentrate through an 80 μm mesh screen. In order to count trapped microzooplankton larger than 80 μm, the material on the screen is washed into a container, transferred to a settling chamber to settle for 24 hours and is then examined with an inverted plankton microscope for counting the microzooplankton. This represented the first sub-set counted.

To obtain the other two sub-sets, the 100 ml concentrate is gently swirled and mixed. Based on the amount of detritus and plankters, a 5 or 10 ml aliquot is taken

and placed in a second settling chamber, with enough buffered formalin solution added to the chamber to bring to a 25 ml volume. After 10 minutes, 10 ml is removed and placed in a third settling chamber. Both of these chambers (representing the 2nd and 3rd sub-sets) are allowed to settle for 24 hours before examination with the inverted plankton microscope. Counts from the third chamber represent mainly the smaller, and lighter non-loricate ciliates and other protozoa that are often covered with silt if not separated in this fashion. Mid-sized microzooplankters are common in the second chamber. Multiplication constants for count determinations are made, with replicate counts averaged on samples coming from the two carboys at each station.

RESULTS

Station Locations

Mean salinities were determined using monthly salinity measurements taken at each of these stations over a 5 year period (Marshall, 1992). The tidal fresh stations were TF5.5 and TF4.2, in the James and York Rivers respectively. The James River station RET5.2 (1.9‰) is oligohaline, with the York River station RET4.3 (9.6‰) mesohaline. The Elizabeth River stations (ER2, ER5) are within meso-polyhaline ranges. All the Chesapeake Bay stations are polyhaline, with mean salinity values for the stations as follows: WE4.2 at the mouth of the York (21.2‰), LE5.5 at the mouth of the James (23.3‰), CB6.1 (19.7 ‰), and CB7.4 (28.3‰).

Seasonal Microzooplankton Abundance

A. Non-loricate ciliates.

This group represents one of the most abundant components of the microzooplankton throughout the year at river and Bay stations. Common genera within this group were *Strombidium* and *Strobilidium* (Oligotricha) and *Didinium* (Holotricha). Found in every sample throughout the year, the greatest abundance of ciliates was from mid-summer (July) to early fall (Figure 2). These concentrations decreased into winter and gradually increased in spring, with high concentrations associated with waters across a broad salinity gradient. Concentrations ranged from a low of 20/L in December at TF4.2 to a high of 19,500/L in June at ER5. The annual monthly mean was 2,518.2/L (Table 1) across all stations, with a December mean low of 272/L and a July high of 7,199/L. In general, lowest mean monthly concentrations occurred at TF4.2 (830/L), but at the other tidal fresh station (TF5.5), the abundance was greater at 2,181/Liter (Figure 3). Highest mean station values were at ER5 and RET4.3 at 5,232 and 4,601/L respectively. Brownlee and Jacobs (1987) reported September counts of non-loricate ciliates for the Choptank and Chester Rivers as 5,600 and 10,000/L respectively, but did not report on this group from their Bay samples. Marshall et al. (1988) found high ciliate counts in Back Bay during winter and spring that ranged from 3-5 x 10^4/L.

B. Tintinnids (loricate ciliates).

The tintinnids had two seasonal periods of maximum growth (Figure 4). These included a spring peak and increased abundance from summer into early fall. Highest concentrations of 12,913 and 11,253/L were recorded for February at CB7.4 and April at WE4.2 respectively (Marshall, 1993). The annual monthly mean was 1,400.1/L (Table 1), ranging from a January low of 216/L to a high of 3,305/L

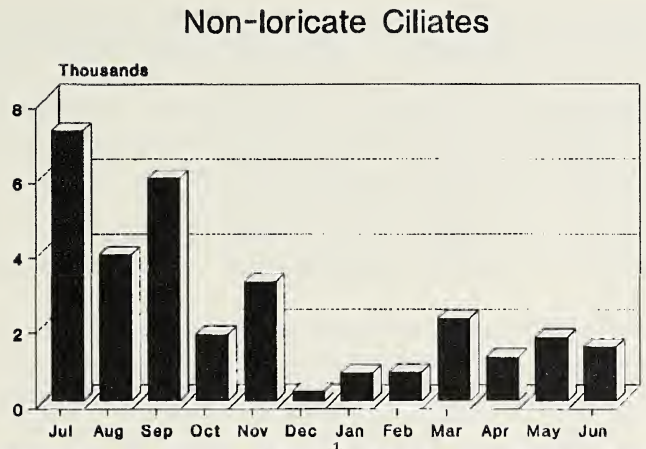

FIGURE 2. Monthly concentration means (No.L^{-1}) of non-loricate ciliates at all stations from July 1992 through 1993.

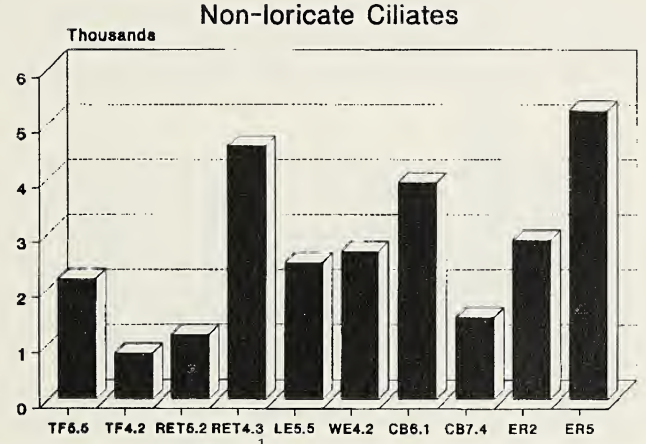

FIGURE 3. Mean concentrations (No.L^{-1}) of non-loricate ciliates for all stations from July 1992 through June 1993.

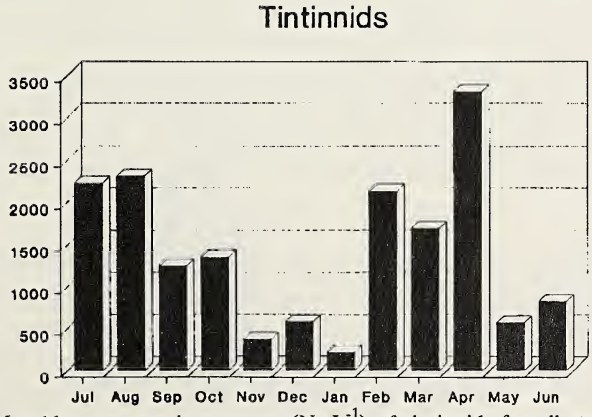

FIGURE 4. Monthly concentrations means (No.L^{-1}) of tintinnids for all stations from July 1992 through June 1992.

in April. The annual station means for tintinnids ranged from 515/L per month at TF4.2 to 2,522/L at CB7.4 (Figure 5). This group was a common constituent in all samples and across a broad salinity gradient throughout the year. The common genera were *Tintinnopsis*, *Tintinnidium*, and *Eutintinnus*. Brownlee and Jacobs (1987) reported the mean concentrations of tintinnids for all stations and dates in the Chesapeake Bay as 160/L, with September counts in the Choptank and Chester Rivers of 17,600 and 4,600/L. They found the tintinnids common year round in the Bay, with peak growth in spring, summer and fall. Marshall et al. (1988) noted tintinnid abundance in Back Bay ranged from 256/L in winter to a high of 36,825/L in spring.

Dolan and Coats (1991) determined the concentrations of ciliates at stations in the upper Chesapeake Bay between the months of April and September. They noted surface abundance ranged from 1,000 to 90,000/L, with maximum numbers occurring in late spring to early summer. Dolan (1991), commented further on these populations, stating the macrophagous microzooplankters (mostly tintinnids and large oligotrichs) ranged between 1-20 x 10^3 organisms/L, with the microphagous components (large scuticociliates and small oligotrichs) between 1-22 x 10^3 organisms/L. The smaller ciliates increased in abundance from April through July, with the tintinnids and larger oligotrichs reaching an earlier peak in June.

C. Nauplii larvae.

A variety of nauplii (mostly copepod larvae) representing different species and stages-in-development were present in the samples. All of these forms were counted within this category. Across all stations, there was a single major development that extended from summer through mid-fall (Figure 6). At the Chesapeake Bay stations there was another pulse that began in February, reached its peak in April, then declined. This development, however, may represent a pattern of an extended spring-fall period of development. Only once during the sampling (December at ER5) were no nauplii found in the samples. The highest counts occurred in September at RET4.3 with 1,418/L (Marshall, 1993). The mean monthly count was 121.7/L (Table 1), with January and September having the lowest and highest values respectively at 17 and 375/L (Figure 6). In general, the tidal fresh stations (TF5.5, TF4.2) had the lowest mean concentrations (47 and 22/L), in contrast to RET4.3 (199/L) and the two stations at the river mouths, LE5.5 and WE4.2 (176 and 173/L), which had the highest abundance (Figure 7). Brownlee and Jacobs (1987) reported a concentration mean of copepod nauplii at 39.2/L for the Chesapeake Bay, with spring and early fall peaks. They also reported nauplii abundance in the Choptank and Chester Rivers for September at 200 and 100/L respectively.

D. Rotifers.

A variety of rotifers were in the samples, including representatives from the genera *Synchaeta, Filinia, Brachionus*, *Keratella*, and *Trichocerca.* Only on 11 occasions were rotifers absent in the samples; concentrations thus ranged from zero to a maximum of 1,915/L in August at TF5.5. The seasonal patterns indicated a spring maximum and a larger summer-fall peak (Figure 8) over a growth period that began in early spring and extended into early fall. Concentrations were greatest from mid-summer through early fall. The river stations indicated more of a

TABLE 1. Mean concentrations and composition percentages of the four microzooplankton components from all stations from July 1992 through June 1993. Composition ranges indicate monthly variations.

Microzooplankton	No./L	Mean %	%Ranges
Ciliates*	2,518.2	59.5	35.2 - 77.1
Tintinnids	1,400.1	33.1	19.6 - 60.5
Rotifers	191.4	4.5	1.3 - 11.4
Nauplii larvae	121.7	2.9	1.2 - 4.4
Total	4,231.4		

*Non-loricate ciliates

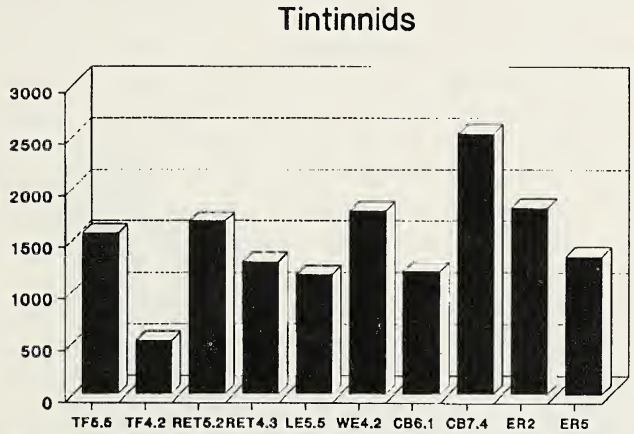

FIGURE 5. Mean concentrations (No.L^{-1}) of tintinnids for all stations from July 1992 through June 1993.

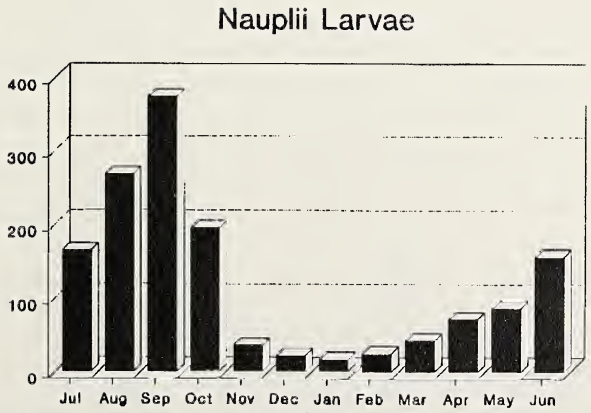

FIGURE 6. Monthly concentration means (No.L^{-1}) of nauplii larvae at all stations from July 1992 through 1993.

bi-modal growth pattern compared to a rather sporadic appearance in the Chesapeake Bay, where growth was more consistent during fall and early winter (Marshall, 1993). The monthly mean was 191.4/L (Table 1), with the lowest (40/L) concentrations in December and the highest (446/L) in August. Highest mean concentrations (420 and 470/L) were associated with two stations, both in the James River (TF5.5, RET5.2), with the lowest concentrations (44/L) at the most saline site, CB7.4 (Figure 9). Brownlee and Jacobs (1987) reported a mean value of 476/L for rotifers in the Chesapeake Bay, with September concentrations of 100 and 500/L for the Choptank and Chester Rivers, and found rotifers more common in the Bay during the colder months. At Back Bay, concentrations were highest in spring at 21/L, but low during other seasons (Marshall et al., 1988).

E. Total Microzooplankton

Seasonal microzooplankton concentrations were greatest in summer followed by fall, spring and winter months (Figure 10). There was a mid-summer maximum that occurred in July (10.0×10^3/L), with high concentrations maintained in August (6.9×10^3/L) and early fall (7.9×10^3/L). The overall monthly concentration mean for the microzooplankton in this study was 4,231.4/L (Table 1). For the Choptank and Chester Rivers in September, Brownlee and Jacobs (1987) reported total concentrations of 24.3 and 15.2×10^3/L respectively. The major component in their samples were tintinnids. A spring pulse of less magnitude, with microzooplankton concentrations lowest during December and January (910 and 1,058/L) was observed. They reported a similar pattern for the total microzooplankton in the Bay, with late summer and fall peaks, with some groups (e.g. tintinnids) having a spring peak at certain stations.

The distribution of these microzooplankton and their abundance at the Bay and river stations were not similar over the sampling area (Figs. 3,5,7,9,11). Highest mean station concentrations were associated with the Chesapeake Bay, RET4.3 in the York and the stations in the Elizabeth River. The highest numbers were at ER5, with mean monthly concentrations of 7,292/L. The lowest figures were at stations in the York tidal fresh waters (TF4.2) at 1,480/L, and the James River (TF5.5, RET5.2) and the Bay station at the mouth of the James (LE5.5). Station TF4.2 consistently had low concentrations of each microzooplankton category. The tintinnids had their lowest abundance in the James River, but were well represented at the other stations. In contrast, the rotifers were more abundant at the James River sites. The nauplii larvae consistently had high concentrations in the Bay.

The non-loricate ciliates composed 59.5% of the microzooplankton, ranging from 35.2% to 77.1% at the different stations (Table 1). The concentrations were below 50% at only two (RET5.2, CB7.4) of the ten stations, with the highest percentage (77.1%) at ER5 in the Elizabeth River. The tintinnids were the second most abundant group, with a range of 19.6% to 60.5% of the total microzooplankton, and a mean concentration of 33.1%. Their highest concentrations of 50.5% and 60.3% were at RET5.2 and CB7.4 respectively (Marshall, 1993), with their lowest values of 20.4%, 21.5%, and 19.6% at stations RET4.3, CB6.1 and ER5 respectively (Marshall, 1993). The nauplii larvae concentrations ranged from 1.2% to 4.4% of the total, with a mean of 2.9% (Table 1). These lowest and highest percentages were at stations TF5.5 and LE5.5 (Marshall, 1993). Concentrations of rotifers did not exceed 11.4%, with this value found at station RET5.2. Rotifers

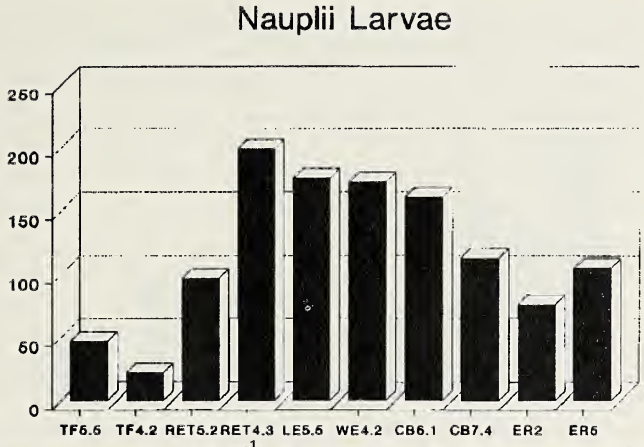

FIGURE 7. Mean concentrations (No.L^{-1}) of nauplii larvae for all stations from July 1992 through June 1993.

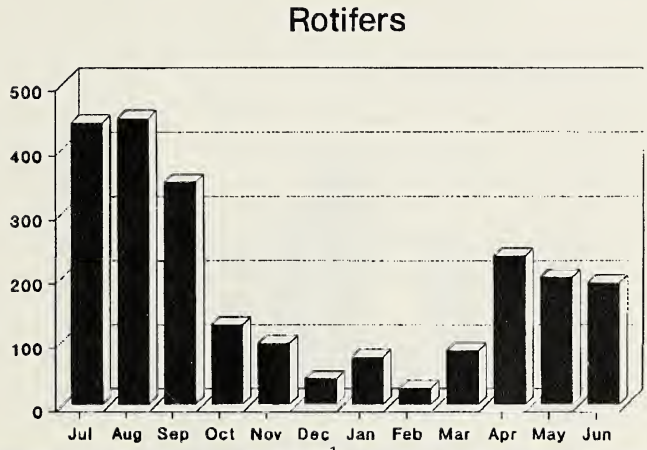

FIGURE 8. Monthly concentrations means (No.L^{-1}) of rotifers for all stations from July 1992 through June 1992.

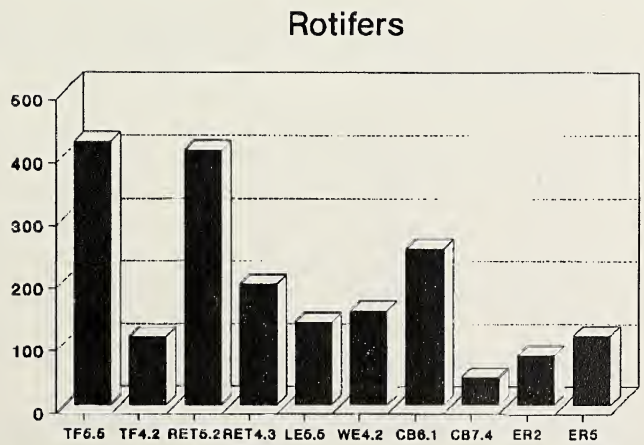

FIGURE 9. Mean concentrations (No.L^{-1}) of rotifers for all stations from July 1992 through June 1993.

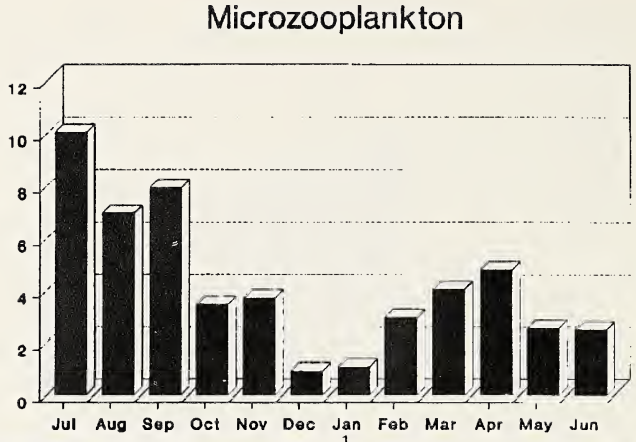

FIGURE 10. Monthly concentrations means (No.L^{-1}) of total microzooplankton for all stations from July 1992 through June 1992.

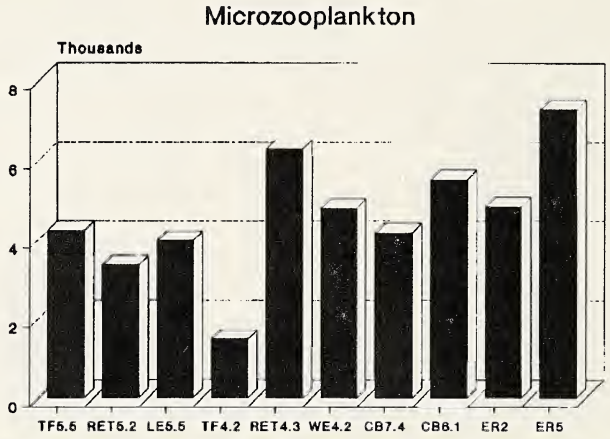

FIGURE 11. Mean concentrations (No.L^{-1}) of total microzooplankton for all stations from July 1992 through June 1993.

represented 4.5% of the total, with a range of 1.3% to 11.4%. The lowest percentages of rotifers were associated with the two Elizabeth River stations (1.8%) and the Bay entrance, at CB7.4 (1.3%).

SUMMARY

The most common and abundant microzooplankton throughout the study were the non-loricate ciliates and tintinnids. They had respectively monthly mean concentrations of 2,518.2 and 1,400.1/L. These two components had the greatest percentage of microzooplankters in the samples. The non-loricate ciliates represented 59.5% of the microzooplankton, with the tintinnids 33.1%. The nauplii larvae had mean monthly concentration of 121.7/L, with rotifers at 191.4/L. In reference to the total microzooplankton composition, the nauplii larvae and the rotifers represented 2.9% and 4.5% respectively of the total.

The seasonal abundance pattern for microzooplankton in the lower Chesapeake Bay indicated a spring and mid-summer to early fall maxima. This

expression is similar to previous findings in the Bay region (Brownlee and Jacobs, 1987; Dolan and Coats, 1991) and elsewhere (Smetacek, 1981; Hargraves, 1981, Capriulo and Carpenter, 1983; Marshall et al., 1988). Individual concentrations for the microzooplankton categories were also within those previously reported for the Bay (Brownlee and Jacobs, 1987; Dolan, 1991; Dolan and Coats, 1991).

The relationship of microzooplankton populations to host and prey associations has been studied in the Chesapeake Bay (Dolan, 1991), however the role microzooplankton play as a consumer and as prey for other organisms has not been clearly defined. The seasonal growth maxima of the microzooplankton components occur from early spring to late fall. These peaks coincide with the development of major phytoplankton pulses within the Bay system (Marshall, 1992). For instance, there are significant floral growth periods occurring during spring, summer and fall, and these include phytoplankters of nano and picoplankton sized cells that are available as food for the microzooplankters. The most dramatic growth of autotrophic picoplankton occurs in summer (June-August), when they increase from background concentrations of 10^6 to 10^9 cells/L. They reach their peak abundance in July, or early August, and consist of mostly cyanobacteria (Marshall, 1992). In addition to the year round presence of the picoplankton (autotrophic and heterotrophic), other smaller phytoplankton exist in abundance in spring (chlorophytes), summer (phytoflagellates, cyanobacteria), and fall (phytoflagellates). One would assume there is currently an adequate supply of phytoplankton year round as a food source for the smaller members within this group. This opinion is supported by Dolan and Coats (1991) who state the ciliate component is not linked to the standing phytoplankton crop, with the ciliates still representing the largest microzooplankton component in the lower Chesapeake Bay.

In conclusion, the lower Chesapeake Bay and the tidal regions of the three rivers in this study contain an abundant microzooplankton community that is dominated by the ciliate protozoa.

ACKNOWLEDGEMENTS

This study is a component of the Chesapeake Bay Monitoring Program that was funded by the Virginia Department of Environmental Quality. G.S. Park is a graduate student in the Department of Biological Sciences who collected and analyzed the samples. The study was conducted in the Old Dominion University Phytoplankton Laboratory under the supervision of H.G. Marshall, who prepared the manuscript and figures. Special thanks is given to Karen Soucek for data entry and to other members of the Old Dominion University Phytoplankton, and Zooplankton Laboratories who helped in collecting the samples.

LITERATURE CITED

Beers, J.R. and G.L. Stewart. 1967. Microzooplankton in the euphotic zone at five locations across the California Current. J. Fisheries Research Board of Canada, 24:2053-2068.

Brownlee, D.C. and F. Jacobs. 1987. Mesozooplankton and microzooplankton in the Chesapeake Bay. *In*: S. Majumdar, L. Hall, and H. Austin (eds.) Contaminant Problems and Management of Living Chesapeake Bay Resources, The Pennsylvania Academy of Science, Easton, Pa., pp.217-269.

Capriulo, G.M. and E.J. Carpenter. 1983. Abundance, species composition and feeding impact of tintinnid micro-zooplankton in central Long Island Sound. Marine Ecol. Prog. Ser. 10:277-288.

Chang, F.H. 1990. Quantitative distribution of microzooplankton off Westland, New Zealand. New Zealand Journal Marine and Freshwater Research, 24:187-195.

Cowles, R.P. 1930. A biological study of the offshore waters of Chesapeake Bay. Bull. Bureau Fisheries, 46:277-381.

Dolan, J.R. 1991. Guilds of ciliate microzooplankton in the Chesapeake Bay. Estuarine, Coastal and Shelf Science, 33:137-152.

Dolan, J.R. and D.W. Coats. 1991. Changes in fine-scale vertical distributions of ciliate microzooplankton related to anoxia in Chesapeake Bay waters. Marine Microbial Food Webs, 5:81-93.

Hargraves, P.E. 1981. Seasonal variations of tintinnids (Ciliophora:Oligotrichida) in Narragansett Bay, Rhode Island, U.S.A. J. Plankton Research, 3:81-91.

Laval-Peuto, M., H. Heinbokel, O. Anderson, F. Rassoulzadegan and B. Sherr. 1986. Role of micro-and nanozooplankton in marine food webs. Insect Science Applications, 3:387-395.

Marshall, H.G., R. Southwick and B. Wagoner. 1988. Seasonal zooplankton composition and abundance patterns in Back Bay, Virginia. Old Dominion University Research Foundation, Special Report for the Commonwealth of Virginia, Dept. of Game and Inland Fisheries, 37 pp.

Marshall, H.G. 1992. Phytoplankton Community. In:Lower Chesapeake Bay Monitoring Program Synthesis Report, 1985-1989. Vol.II. Old Dominion University Research Foundation, Tech. Rpt. Norfolk, Va., 162 pp.

Marshall, H.G. 1993. Microzooplankton in the lower Chesapeake Bay, and the tidal James, York, and Elizabeth Rivers: July 1992-June 1993. Special Report. Old Dominion University Research Foundation, Norfolk, Va., 62 pp.

Morse, D.C. 1947. Some observations on seasonal variation in plankton population, Patuxent River, Maryland, 1943-1945. Chesapeake Biology Laboratory, 65:1-31.

Rassoulzadegan, F. and R. Sheldon. 1986. Predator-prey interaction of nanozooplankton and bacteria in an oligotrophic marine environment. Limnology and Oceanography, 31:1010-1021.

Sieburth, J. McN., V. Smetacek and J. Lenz. 1978. Pelagic ecosystem structure:heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnology and Oceanography, 23:1256-1263.

Smetacek, V. 1981. The annual cycle of protozooplankton in the Kiel Bight. Marine Biology, 63:1-11.

Whaley, R. and W. Taylor. 1968. A plankton survey of the Chesapeake Bay using a continuous underway sampling system. Chesapeake Bay Institute, Tech. Report. No. 36, 89 pp.

Wolfe, J., B. Cunningham, N. Wilderson and J. Barnes. 1926. An investigation of the microplankton of Chesapeake Bay. Journal of the Elisha Mitchell Scientific Society, 42:25-54.

Virginia Journal of Science
Volume 44, Number 4
Winter 1993

A Statistical Result Derived from Mechanical Equilibrium

J. N. Boyd and **P. N. Raychowdhury**,
Department of Mathematical Sciences,
Virginia Commonwealth University,
Richmond, VA 23284-2014

ABSTRACT

The statistical formula SST = SSM + SSE is central to the ideas of linear regression. If values (x) of a random variable X and corresponding values (y) of a second random variable Y are assumed to have the linear relationship $y = b_1x + b_0$ as obtained by the least-squares fit, then the statistical formula partitions the total variation of the observed values of y from their mean (SST) into two summands which represent the variation of the predicted values of y from the mean (SSM) and the variation of the observed values from the corresponding values predicted by the regression (SSE).

INTRODUCTION

Let us suppose that we have n data points (x_i, y_i), i = 1,2,3, ..., n. The first coordinate x is taken to be a value of an independent random variable X and y is taken to be the corresponding value of a dependent or response random variable Y. The line ℓ which has equation

$$y = b_1x + b_0 \tag{1}$$

and minimizes the sum

$$S = \sum (y_i - b_1 x_i - b_0)^2 \tag{2}$$

is the least-squares regression line for the data. Since sums will always run from 1 to n over all data points, we omit the limits of summation in our notation.

Let $\hat{y}_i = b_1 x_i + b_0$ denote the value of the response variable predicted by the model when $x = x_i$. In general, $\hat{y}_i \neq y_i$ and we denote the difference by $r_i = y_i - \hat{y}_i$ for each i. This difference is called the i-th residual for the model and sum 2 can be rewritten as

$$S = \sum r_i^2 \ . \tag{3}$$

For a further bit of notation, we let $\bar{y} = \sum y_i / n$ denote the mean of the observed values of y.

THE STATISTICAL FORMULA

Let $SST = \sum (y_i - \bar{y})^2$, $SSM = \sum (\hat{y_i} - \bar{y})^2$, and $SSE = \sum (y_i - \hat{y_i})^2$. The symbols SST, SSM, and SSE stand for "total sum of squares," "model sum of squares," and "error sum of squares." The formula which we wish to derive is a significant one:

$$SST = SSM + SSE. \tag{4}$$

Its derivation is not generally given in introductory statistics texts or in texts devoted to the methods of statistics.

AN EQUIVALENT PROBLEM

Let us begin our derivation by noting that $y_i - \bar{y} = (\hat{y_i} - \bar{y}) + (y_i - \hat{y_i})$ and that $(y_i - \bar{y})^2 = (\hat{y_i} - \bar{y})^2 + (y_i - \hat{y_i})^2 + 2(\hat{y_i} - \bar{y})(y_i - \hat{y_i})$. After summing both sides of the last equation from 1 to n, it becomes clear that our problem is equivalent to showing that

$$T = \sum (\hat{y_i} - \bar{y})(y_i - \hat{y_i}) \tag{5}$$

is zero.

Letting $y_i = \hat{y_i} + r_i$ in equation 5, we can rewrite that equation as

$$T = \sum (\hat{y_i} - \bar{y}) r_i \ . \tag{6}$$

The computations to develop the regression equation $y = b_1x + b_0$ make it clear that $(\bar{x}, \bar{y})$ satisfy the equation where $\bar{x}$ and $\bar{y}$ are the means of the observed x_i and y_i, respectively. Thus equation 6 becomes

$$T = b_1 \sum (x_i - \bar{x}) r_i \ . \tag{7}$$

THE LINE IN MECHANICAL EQUILIBRIUM

Let us imagine that regression line ℓ is pivoted at $(\bar{x}, \bar{y})$ and is free to rotate about the axis perpendicular to the xy-plane at $(\bar{x}, \bar{y})$. The line together with several data points is shown in Figure 1.

If $b_1 = 0$, the derivation of equation 4 is trivial. Therefore, we assume that $b_1 \neq 0$. Then equation 4 is true if and only if $\sum (x_i - \bar{x}) r_i = 0$.

Let us imagine a configuration of n springs above and below line ℓ. The springs may be stretched parallel to the y-axis and the spring constant for each is $k > 0$. Imagine that the i-th spring has one end fixed on the line $x = x_i$ and that the other end is free but constrained to stay on the line $x = x_i$. When the spring is unstretched, this free end is located at (x_i, y_i). Stretch the spring a distance $|r_i|$ and attach the free end to line ℓ. The situation is illustrated in Figure 2. We imagine that at the point of attachment there is a frictionless ring which can slide on ℓ so that the springs remain parallel to the y-axis if the line rotates through a small angle about $(\bar{x}, \bar{y})$.

The elastic potential energy for the configuration of springs is $\sum \frac{k}{2} r_i^2$. Since the line is the least-squares regression line, $\sum r_i^2$ and the elastic potential energy must both be minima, and the line is in stable mechanical equilibrium.

The conditions of equilibrium are that the algebraic sum of forces in the y-direction is zero and that torques about the axis at $(\bar{x}, \bar{y})$ must sum to zero.

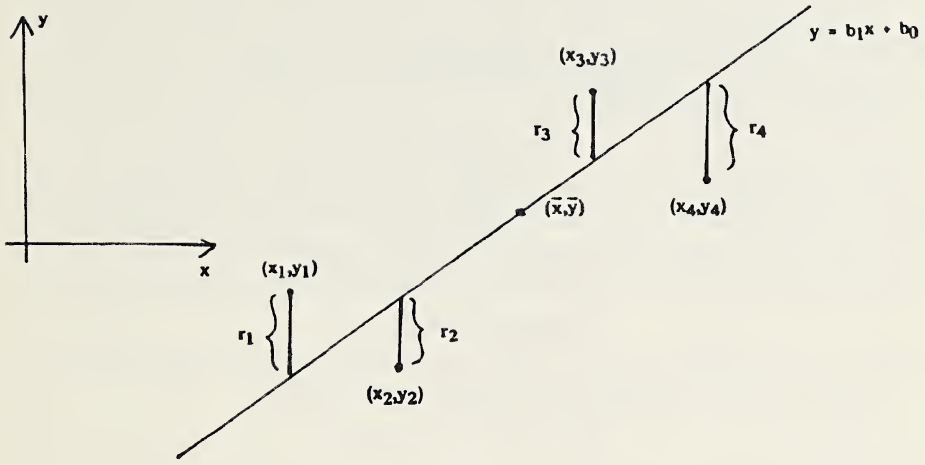

FIGURE 1. The Geometry of Line ℓ.

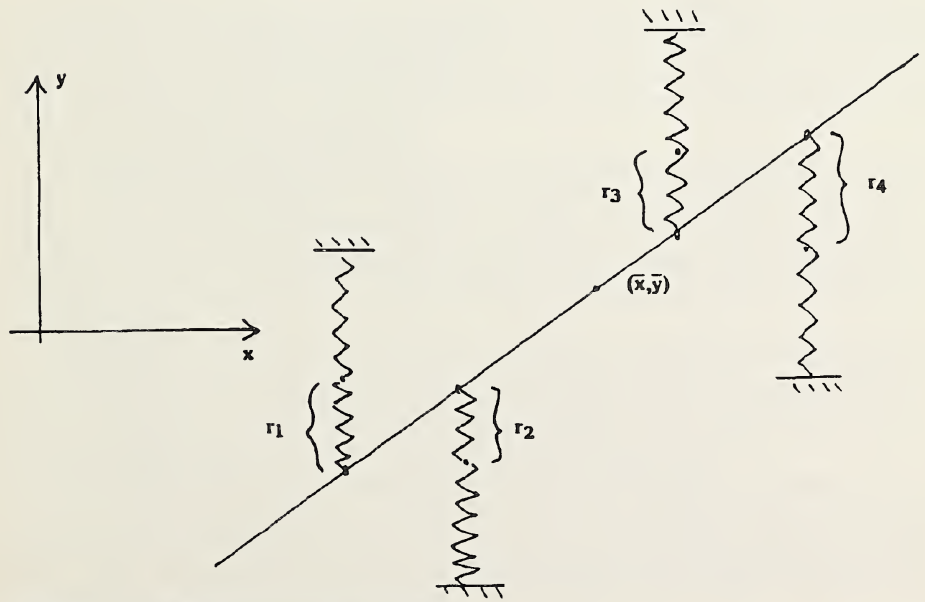

FIGURE 2. Line ℓ in Mechanical Equilibrium.

The first condition tells us that $\sum k r_i = 0$ since the force exerted by the i-th spring is kr_i. Thus $\sum r_i = 0$. This result that the residuals must sum to zero is a bonus for us.

The second condition gives us the result we set out to obtain. Since the i-th torque is computed by force times lever arm or $k r_i (x_i - \bar{x})$, we have $\sum k (x_i - \bar{x}) r_i = 0$. Division by k yields $\sum (x_i - \bar{x}) r_i = 0$.

Thus SST = SSM + SSE.

CONCLUSION

The physical picture of the least-square regression line is a pleasing one. The line is under tension and is in equilibrium. After small displacements, the line tends to return to its least-square configuration. The model is stable. The statistical meaning of the formula derived is well known (Moore and McCabe, 1993).

LITERATURE CITED

Moore, D. S. and McCabe, G. P. 1993. Introduction to the Practice of Statistics. NewYork: W.H. Freeman and Company, p. 656.

ANNOUNCEMENTS

The Barbara J. Harvill Botanical Research Fund for Floristic Research in Virginia

The Barbara J. Harvill Botanical Research Fund was endowed by friends and family of the late Barbara J. Harvill to encourage floristic work in Virginia. It provides small grants to botanists without an institutional base of support for such work. Most of the awards requested to date have been for mileage costs related to field work, but other expenses, such a mileage costs for visits to herbaria, lodging, and certain kinds of field equipment (plant presses, for instance) can also be covered. Please send your letter of application to Donna M. E. Ware, Curator, Herbarium, Department of Biology, College of William and Mary, Williamsburg, VA 232187.

AAAS Seeks Information for Resource Directory

The American Association for the Advancement of Science Project on Science, Technology, and Disability invites scientists and engineers with disabilities to be included in the third edition of the Resource Directory of Scientists and Engineers with Disabilities. Potential candidates for the directory must hold, or be working toward, a degree in a scientific, engineering, or medical discipline, or currently be employed in a scientific field.

Funded by the National Science Foundation, the project's Resource Directory of Scientists and Engineers with Disabilities has assisted hundreds of individuals enter and advance in scientific disciplines. The directory helps to connect persons with disabilities and their families with professors, teachers, and counselors who can serve as role models and mentors.

The Resource Directory lists scientist, mathematicians, and engineers from all parts of the country with their disciplines, degrees, and disabilities. Individuals include professionals who were born with a disability, and those who acquired their disability mid-career. Persons listed in the directory are also asked to consult for academia, government agencies, and industry as well as serve on peer review panels and symposia.

Established in 1975, the AAAS Project on Science, Technology, and Disability has sought and shared expert advice from scientists and engineers with disabilities. Since the passage of the Americans with Disabilities Act (ADA), the AAAS Resource Directory has become a valuable source of expertise.

To be included in the directory, or for more information, please contact Laureen Summers, Program Associate; or Patricia A. Thompson, Editorial Specialist, AAAS Project on Science, Technology, and Disability, AAAS, 1333 H Street, N.W., Washington, DC 20005, or call 202-326-6645 (V/TDD). Information can also be sent via fax to 202-371-9849.

HORSLEY CANCER RESEARCH FUND

The research committee of the Virginia Academy of Science has evaluated the proposals for the Horsley Cancer Research Fund and recommends the funding of the following proposals:

"Transcription"Factors and Cancer" $4,000.00
Dr. David R. Bevan
Dept. of Biochemistry and Anaerobic Microbiology
Virginia Tech
Blacksburg, VA 24061

"Breast Cancer Cell Growth Regulation By Glycosphingolipids and Sphingolipid Breakdown Products" $4,000.00
Dr. Thomas W. Keenan
Dept. of Biochemistry and Anaerobic Microbiology
Virginia Tech
Blacksburg, VA 24061

"In-Vitro and In-Vivo Effects of Tamoxifen on Wound Healing" $2,328.75
Drs. John S. Mancoll & Brad K. Lewis
Dept. of Surgery
Eastern Virginia Medical School
825 Fairfax Avenue
Norfolk, VA 23507

Total . $10,328.75

Thomas O. Sitz, Ph.D.
Chairman, Research Committee
Virginia Academy of Science
(703) 231-4970

NECROLOGY

Dan B. DeLury, a member of Virginia Tech's statistics faculty in the Department of Agricultural Economics from 1945-1947, died recently. At the time of his death he was professor emeritus of the University of Toronto (Canada). Dr. DeLury, jointly with Dr. Boyd Harshbarger (founder and first head of Virginia Tech's Statistics Department), was instrumental in laying the foundation for statistics to become a separate department at Virginia Tech in 1949. His paper "The analysis of Latin squares when some observations are missing" received the J. Shelton Horrley Research Award of the Virginia Academy of Science in 1946.

NOTES

NOTES

NOTES

MEMBERSHIP

Membership in the Academy is organized into sections representing various scientific disciplines as follows:

1. Agriculture, Forestry and Aquaculture
2. Astronomy, Mathematics and Physics
3. Microbiology and Molecular Biology
4. Biology
5. Chemistry
6. Materials Sciences
7. Biomedical and General Engineering
8. Geology
9. Medical Sciences
10. Psychology
11. Education
12. Statistics
13. Aeronautical and Aerospace Sciences
14. Botany
15. Environmental Science
16. Archaeology
17. Computer Science
18. Geography

Annual Membership Dues - Includes subscription to **Virginia Journal of Science**

Student	$ 10.00
Regular - Individual	25.00
Contributing - Individual	30.00
Sustaining - Individual	50.00
Sustaining - Institution	100.00
Business - Regular	100.00
Business - Contributing	300.00
Business - Sustaining	500.00
Life - Individual	300.00

VIRGINIA ACADEMY OF SCIENCE

APPLICATION FOR MEMBERSHIP

Date _____ Name (Please Print) _____

Phone (____)_____ E-mail _____ FAX(____) _____

Address _____

City_____ State_____ Zip _____

Institution or Business _____

Position — Title _____

Fields of Interest — Section No.(s) _____ First No. indicates major interest

Class of Membership Desired _____

Contacted by: _____

Make check payable to **VIRGINIA ACADEMY OF SCIENCE** and send to:
Department of Biology, University of Richmond, Richmond, VA 23173

Instructions to Authors

All manuscripts and correspondence should be addressed to the Editor. The Virginia Journal of Science welcomes for consideration original articles and short notes in the various disciplines of engineering and science. Cross-disciplinary papers dealing with advancements in science and technology and the impact of these on man and society are particularly welcome. Submission of an article implies that the article has not been published elsewhere while under consideration by the Journal.

Three complete copies of each manuscript an figures are required. It is also suggested that authors include a 5.25 diskette in IBM compatible format containing a text file (ASCII) of the manuscript. Original figures need not be sent at this time. Authors should submit names of three potential reviewers. All manuscripts must be double-spaced. **Do not** use special effects such as bold or large print.

The title, author's name, affiliation, and address should be placed on a cover page. An abstract (not to exceed 200 words) summarizing the text, particularly the results and conclusions, is required. The text should follow the general format used by professional journals in the author's discipline. Literature cited in the text should follow the name-year format: (McCaffrey and Dueser, 1990) or (Williams et al., 1990). In the Literature Cited section at the end of the article, each reference should include the full name of the author(s), year, title of article, title of journal (using standard abbreviations), volume number and first and last page of the article. For a book, include author(s), year, title, pages or number of pages, publisher and city of publication. Examples:

McCaffrey, Cheryl A. and Raymond D. Dueser. 1990. Plant associations of the Virginia barrier islands. Va. J. Sci. 41:282-299.

Spry, A. 1969. Metamorphic Textures. Pergamon Press, New York. 350 pp.

Each figure and table should be mentioned specifically in the text. All tables, figures and figure legends should be on a separate pages at the end of the text.

Multiple author papers are required to have a statement in the acknowledgements indicating the participation and contribution of each author.

After revision and final acceptance of an article, the author will be required to furnish two error-free copies of the manuscript: 1) typed copy, single spaced, with tables and figure captions at the end of the document, and one set of original figures, each identified on the back by figure number and author's name; 2) a 5.25 diskette in an IBM compatible format containing the text file, tables and figure legends.

Authors will be allowed 15 printed pages (including figures) free, but payment of $50 per page will be charged for the l6th and subsequent pages.

Virginia Academy of Science
Department of Biology
University of Richmond, Virginia 23173

Address Correction Requested